Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Combination of a urease inhibitor and a plant essential oil to control coliform bacteria, odour production and ammonia loss from cattle waste

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Keywords
cattle manure, coliforms, pathogens, plant oils, urease inhibitor.

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2006/0322: received 7 March 2006, revised 4 May 2006 and accepted 15 May 2006

doi:10.1111/j.1365-2672.2006.03120.x

Abstract

Aim: To evaluate urea hydrolysis, volatile fatty acid (VFA) production (odour) and coliforms in cattle waste slurries after a urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT) and a plant oil component (thymol) were added.
Methods and Results: Faeces from cattle fed a diet of 70% corn silage and 30% alfalfa haylage, urine and distilled water in the ratio 50 : 35 : 15 were blended at high speed for 1 min. Triplicate aliquots of 750 ml were amended with NBPT plus or minus thymol and reblended for 1 min, and were poured into 1·6 l wide-mouth jars covered 90% with a lid. After 56 days, thymol (2000 mg kg^{-1} waste) in combination with NBPT (80 mg kg^{-1} waste) retained 5·2 g of an initial 9·2 g of urea in cattle waste slurries, compared with less than 1 g of urea retained when NBPT was the only additive ($P < 0·05$). Another experiment using excreta from cattle fed 76·25% high moisture corn, 19·25% corn silage and a 4·5% supplement, blended at a low speed, gave a similar response with urea hydrolysis; and the two treatments, thymol alone and thymol in combination with NBPT, reduced VFA production ($P < 0·01$) and eliminated all coliform bacteria by day 1. A third experiment indicated coliforms disappeared in the no addition treatment after 8 days; however, they were viable at $6·6 \times 10^4$ CFU g^{-1} waste beyond 35 days in the NBPT treatment.
Conclusions: Thymol supplements the effect of NBPT by increasing the inhibitory period for hydrolysis of urea in cattle waste slurries and nitrogen retention in the waste.
Significance and Impact of the Study: Thymol and NBPT offer the potential to reduce odour and pathogens in cattle manure, and increase the fertilizer value.

Introduction

A recent report from the National Research Council of the National Academies in the USA ranked ammonia and odour as their highest concerns for emissions from confined animal feeding operations (CAFOs; National Research Council of the National Academies 2002). Most of the ammonia emissions from livestock wastes originate from hydrolysis of urea (Van Horn *et al.* 1996; Bierman *et al.* 1999; Varel *et al.* 1999). Beef cattle raised in a CAFO excrete approximately 60% to 80% of their nitrogen in urine and the remainder in faeces. Urine contains up to 97% urea nitrogen, which is readily hydrolysed by microbial urease to ammonia shortly after excretion. Ammonia emissions in Europe have increased by more than 50% during the past 30 years. Livestock production has been identified as the primary contributor to this increase (Pain *et al.* 1998; McCrory and Hobbs 2001).

Urease inhibitors have been used successfully, on a short-term basis (2 weeks), to prevent ammonia emissions from urea-based fertilizers when they are applied to soil (Byrnes and Freney 1995). Similarly with cattle feedlot manure, the urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT) has the potential to prevent

ammonia emission and retain urea nitrogen in the manure (Varel *et al.* 1999; Shi *et al.* 2001; Parker *et al.* 2005). Urease inhibitors bind to the active nickel site of the enzyme and prevent urea hydrolysis to ammonia and carbon dioxide (Mobley *et al.* 1995). However, McCrory and Hobbs (2001) concluded that urease inhibitors are too expensive and easily broken down or inactivated to bring any economic or practical benefit to livestock producers. Further research is needed to develop products that prolong the effectiveness of urease inhibitors in manure environments.

Efforts to control ammonia emissions from CAFOs should be complementary to controlling odour emissions. Recently, Coates *et al.* (2005) have demonstrated that Fe(III) supplementation to swine waste can be used to stimulate micro-organisms to metabolize the malodorous VFA compounds once they are produced. Another approach may be amendments with antimicrobial plant oil components, in particular, the phenolic oils thymol, carvacrol and eugenol, which as a class of chemicals, are the more potent (Burt 2004). They have been effective in preventing VFA production and reducing coliform bacteria in cattle and swine waste slurries (Varel and Miller 2001; Varel 2002; Varel and Miller 2004). Thymol is the least expensive of the three and was used in these studies. Therefore, combining an antimicrobial additive with NBPT may prolong the inhibitory period of the urease inhibitor. The objectives of this study were to evaluate the two additives, NBPT and thymol, individually and in combination for their ability to prevent urea hydrolysis and reduce VFA production and coliform bacteria in waste slurries from cattle fed various growing-finishing diets.

Materials and methods

Chemicals

Chemicals were purchased from Sigma Chemical Company (St Louis, MO, USA), with the exception of NBPT, which came from Agrotain International (St Louis, MO, USA). Purity of thymol was 98%.

Cattle waste slurries

Three experiments were conducted in which the diets, blending speed and NBPT concentration were varied as indicated in the data presented. Diet played an important role because of the amount of corn (starch) excreted in the faeces. Waste slurry blending speed (low or high) was important because it either completely homogenized the corn granule making the starch readily accessible to the microbes (high speed), or the corn granule remained intact (low speed). The concentration of NBPT was significant (20 or 80 mg kg^{-1} of slurry) because at the lower concentration (experiment 3), coliforms remained viable for a longer period than the no NBPT treatment.

Cattle wastes were processed similarly to earlier studies (Varel and Miller 2001, 2004). Briefly, faecal waste, within 15 min of being excreted, was randomly collected from pens of 40 animals fed various diets indicated in the data presented. Cattle urine was collected from catherized animals. Urea concentration in the urine varied considerably; therefore 3 g urea kg^{-1} of urine was added to ensure urea was present in these experiments. Just prior to use, a desired amount of NBPT was added to the urine used in the two treatments containing NBPT. Cattle faeces, urine with NBPT and distilled water in the ratio of 50 : 35 : 15 (weight basis) were blended on high or low speed (Waring Inc., New Hartford, CT, USA) for 1 min. Similarly, another batch of slurry was prepared with urine not containing NBPT. After these two batches of slurry were prepared, two samples of 2250 ml from each batch were obtained, thymol (final concentration 2000 mg kg^{-1} slurry) was added to two samples and all four samples were reblended for 1 min. Each 2250 ml sample was then divided into three-750 ml aliquots and poured into a 1·6-l volume jar (17 cm tall, 13·5 cm diameter and 10 cm opening). Plastic lids provided with the jars were used to cover approximately 90% of the jar opening to prevent moisture loss over the 48–56 days experimental periods. Thus, four treatments, no additions, NBPT, NBPT plus thymol and thymol were established, for each of the three diets evaluated. Each treatment was replicated in triplicate. Once the treatments were established, they were immediately sampled (within 30 min); these analyses were considered time, 0. All jars were left stationary at room temperature (21°C) and the contents were periodically sampled.

Analytical methods

A 15-ml waste slurry sample was collected from each jar after briefly stirring the contents with the sampling-pipette. The sample was mixed with 15 ml of 0·5 N H_2S0_4, centrifuged at 2000 *g* for 20 min at 4°C, and stored at −20°C until analysed (Varel and Miller 2000). Short-chain VFA (acetate, propionate, butyrate, valerate, isobutyrate, isovalerate) were determined in an aliquot from the acidified sample as previously described (Varel 2002). Coliform bacteria were enumerated from the contents of the jar with 3M Petrifilm *Escherichia coli* count plates (3M Microbiology Products, St Paul, MN, USA) according to previous methods (Varel and Miller 2001). Manure slurry pH was obtained from the contents in the jars using a combination pH electrode and pH meter. Urea was determined using a modification of the Sigma Blood

Urea Nitrogen kit (BUN, procedure no. 535; Sigma-Aldrich Chemicals, St Louis, MO, USA). Briefly, samples were diluted 10-fold and 20 μl was added to 300 μl BUN acid and 200 μl BUN colour in a glass test tube. Samples with reagents were boiled for 10 min and immediately transferred to cold-water bath for 5 min. A 300 μl aliquot of each sample was transferred to a well in a 96-well microtitre plate. Absorbance at 515 nm was read using a Bio-Tek Ceres UV900C microplate reader (Bio-Tek, Winooski, VT, USA) and urea concentration was determined from linear regression to a standard curve. Ammonia was determined using a modification of Sigma U N kit (procedure no. 640; Sigma). Standards and samples were diluted 10-fold and 5 μl was transferred to a well in a 96-well microtitre plate. This was followed by additions of 50 μl phenol nitroprusside, 50 μl alkaline hypochlorite and 250 μl distilled water. Colour was allowed to develop for 20–30 min at room temperature. Absorbance at 620 nm was measured using a Bio-Tek Ceres UV900C microplate reader.

Statistical analysis

Data were analysed as a split plot in time with GLM procedure of SAS (1996). Results are the means ± SD of triplicate samples. Differences between treatments were determined by least square means ($P < 0{\cdot}05$).

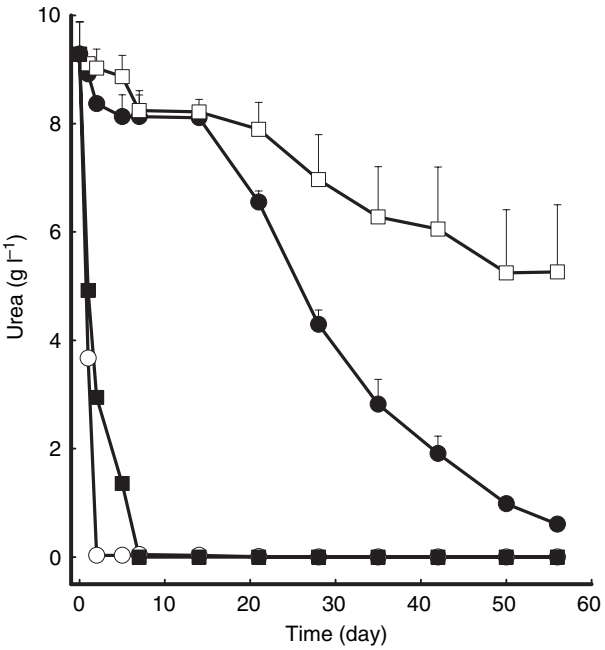

Figure 1 Effect of NBPT and thymol treatments on the hydrolysis of urea in stored cattle waste slurries, which were blended at high speed. Diet of the cattle was 70% corn silage and 30% alfalfa haylage. Treatments included: ○ no additions, ● NBPT 80 mg kg^{-1}, ■ thymol 2000 mg kg^{-1} and □ NBPT 80 mg kg^{-1} plus thymol 2000 mg kg^{-1}.

Results

After 56 days, the thymol in combination with NBPT treatment retained 5·2 kg^{-1} of an initial 9·2 kg^{-1} urea (57%) in a waste slurry from cattle fed 70% corn silage and 30% alfalfa haylage blended at high speed (Fig. 1). This compares with less than 1 kg^{-1} of urea retained when NBPT was the only additive ($P < 0{\cdot}05$). Thymol by itself had little to no inhibitory effect ($P > 0{\cdot}85$) on urea hydrolysis; all was hydrolysed by day 7.

The same treatments imposed on a waste slurry from cattle fed a typical growing-finishing diet (76·25% high moisture corn, 19·25% corn silage, 4·5% supplement consisting of protein, vitamins, minerals and monensin), with the exception that the blending was at low speed (to prevent corn granule breakage), gave a similar urea hydrolysis response. More urea was retained in the treatment containing thymol and NBPT (1·50 g kg^{-1}) than the treatment with NBPT by itself (0·75 g kg^{-1}) for 42 days ($P < 0{\cdot}05$); however, these treatments were not different at 48 days ($P > 0{\cdot}12$; Fig. 2a). Production of VFA in these slurries were the same for the two treatments, which contained thymol (Fig. 2b). The VFA rose from 30 to 40 mmol l^{-1} in these two slurries containing thymol, as opposed to the other two treatments (no addition and NBPT), in which approximately 70 mmol l^{-1} VFA accumulated (30–100 mmol l^{-1}) by 7 days ($P < 0{\cdot}01$). The NBPT had no effect on VFA production. Treatment pH for these slurries is illustrated in Fig. 2c. Coliform bacteria in these slurries were eliminated in the two treatments containing thymol, immediately after thymol was added (Table 1). The no addition treatment initially contained $1{\cdot}0 \times 10^5$ CFU ml^{-1}, which increased to $65{\cdot}0 \times 10^5$ CFU ml^{-1}after 7 days, before declining to approximately the initial concentration by 48 days ($0{\cdot}7 \times 10^5$ CFU ml^{-1}). The NBPT treatment produced a similar response to that of the no addition treatment.

A third experiment was conducted in which the treatments were imposed on a waste slurry obtained from cattle fed a diet of 70% high moisture corn, 25·5% corn silage and 4·5% supplement (same as before), and also blended at high speed (no visible corn granules remained). In this experiment, the NBPT concentration was reduced to 20 mg kg^{-1} slurry. The urea hydrolysis and VFA accumulation results in this experiment (data not shown) were similar to the previous experiment as presented in Fig. 2. However, the pH values for the no addition and NBPT treatments were below 5·0 from days 7 to 21 and days 3 to 7, respectively (Fig. 3). No viable coliform bacteria were observed in the no addition treat-

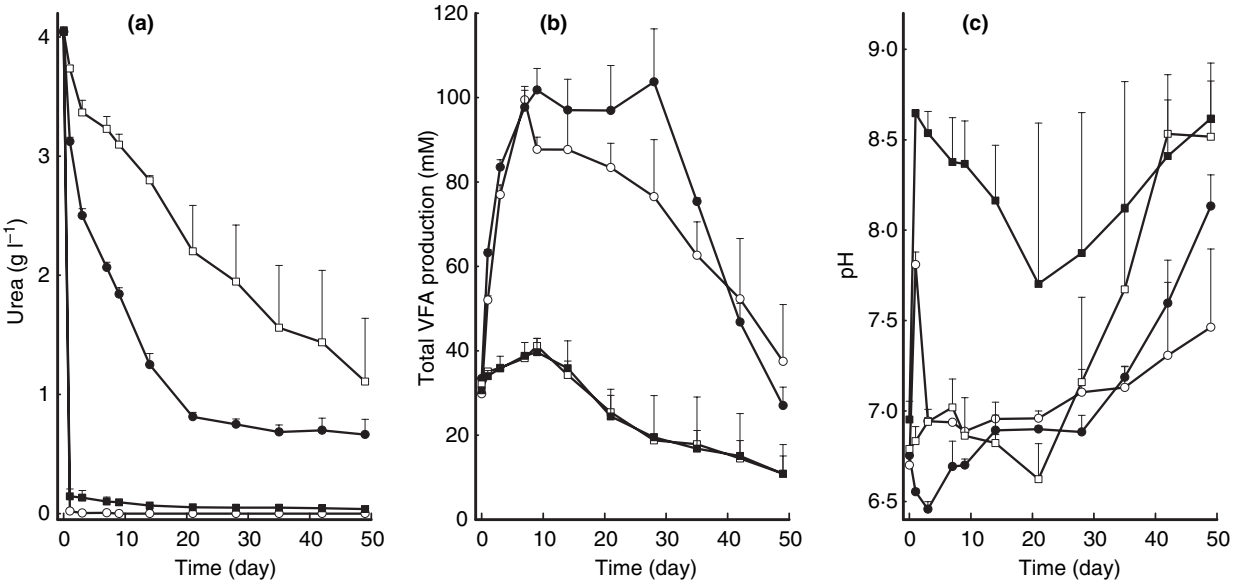

Figure 2 Effect of NBPT and thymol treatments on the hydrolysis of urea (a), VFA (b) and pH (c) in stored cattle waste slurries, which were blended at slow speed. Diet of the cattle was 76·25% high moisture corn, 19·25% corn silage and a 4·5% supplement. Treatments included: ○ no additions, ● NBPT 80 mg kg^{-1}, ■ thymol 2000 mg kg^{-1} and □ NBPT 80 mg kg^{-1} plus thymol 2000 mg kg^{-1}.

Table 1 Effect of thymol, NBPT, or a combination of both on total coliforms in cattle waste slurries incubated anaerobically*

	Coliform bacteria (×10^5 cells ml^{-1})†			
Time (day)	No additions (0)	Thymol (2000‡)	NBPT (80‡)	Thymol (2000‡) + NBPT (80‡)
0	1·0	0	2·0	0
1	1·3	0	13·7	0
3	27·5		48·3	
7	65·0		49·9	
14	23·0		2·0	
28	5·9		2·7	
42	1·7		0·4	
48	0·73		0·53	

*Diet (%) is high moisture corn (76·25), corn silage (19·25) and supplement (4·5); slow speed blending.
†Detection limit is 1 × 10^2 cells ml^{-1}; means are from triplicate jars.
‡mg kg^{-1}.

ment at 7 days and thereafter (Table 2). However, in the NBPT treatment, coliform bacteria remained viable through 49 days.

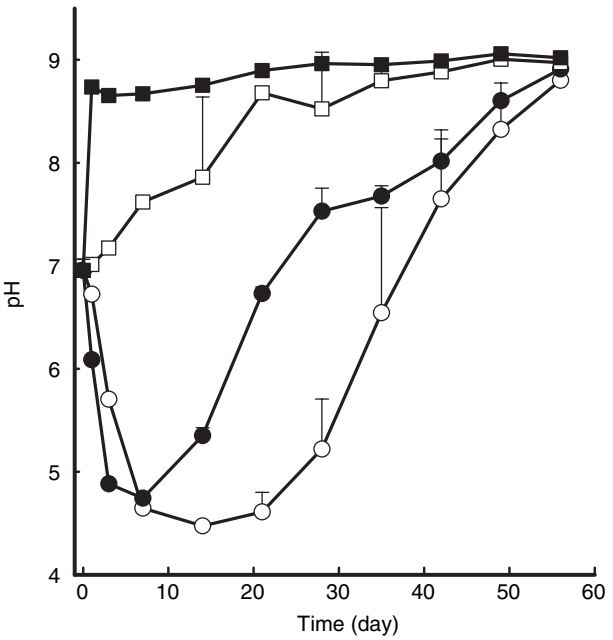

Figure 3 Effect of NBPT and thymol treatments on pH in stored cattle waste slurries, which were blended at high speed. Diet of the cattle was 70% high moisture corn, 25·5% corn silage and a 4·5% supplement. Treatments included: ○ no additions, ● NBPT 80 mg kg^{-1}, ■ thymol 2000 mg kg^{-1} and □ NBPT 80 mg kg^{-1} plus thymol 2000 mg kg^{-1}.

Discussion

Results from this study indicate that thymol has a supplemental effect to the urease inhibitor, NBPT, by prolonging the time before the urea in cattle waste slurry is hydrolysed by microbial urease. This offers the potential to curtail some of the ammonia emissions from cattle feedlots, which can be 50–75% of the nitrogen excreted (Van Horn *et al.* 1996; Bierman *et al.* 1999). When McCrory and Hobbs (2001) wrote their review, they were likely correct that urease inhibitors were too expensive

Table 2 Effect of thymol, NBPT, or a combination of both on total coliforms in cattle waste slurries incubated anaerobically*

Time (day)	Coliform bacteria ($\times 10^5$ CFU ml^{-1})†			
	No additions (0)	Thymol (2000‡)	NBPT (20‡)	Thymol (2000‡) + NBPT (20‡)
0	11·6	0	16·3	0
1	7·3	0	36·3	0
3	10·7		61·3	
7	0		1·3	
14	0		3·2	
28	0		0·74	
42	0		0·13	
49	0		0·003	

*Diet (%) is high moisture corn (70), corn silage (25·5) and supplement (4·5); fast speed blending.
†Detection limit is 1×10^2 cells ml^{-1}; means are from triplicate jars.
‡mg kg^{-1}.

and easily degraded to bring any economic or practical benefit to livestock producers. Today, however, NBPT is being produced more economically. Agrotain International estimates the cost for NBPT to treat one metric ton of cattle manure slurry at 80 ppm or 80 mg kg^{-1}as we have performed in this study, would be approximately 44 cents (US dollar; personal communication, Allen Sutton, Agrotain International, Corydon, KY, USA). Therefore, price is likely not the key issue for NBPT use, in particular if the concentration of retained urea nitrogen in treated manure slurries is double or triple that of untreated slurry. It is assumed the nitrogen-enhanced manure slurry would be worth more than 44 cents per metric ton based upon the rapidly increasing nitrogen fertilizer costs, which have paralleled the energy market. Parker *et al.* (2005) also concluded that the use of NBPT for reducing ammonia emissions from cattle feedlots looks promising.

The key issue with NBPT today is whether or not effectiveness can be maintained over a period longer than 4–11 days as previously demonstrated with laboratory studies (Varel 1997). Increasing the concentration of NBPT to 80 mg kg^{-1} in the current study (Fig. 1) prevented most of the urea from being hydrolysed up to 14 days, but then a gradual hydrolysis began and continued to day 56. We have treated manure slurries with NBPT at 160 mg kg^{-1}slurry and have found the higher concentration has little effect on the rate at which urea is hydrolysed (data not shown). This suggests that microorganisms degrade NBPT, supported by Byrnes and Freney 1995, and an antimicrobial chemical may be necessary to prolong initial inhibitory activity of NBPT.

Field studies indicate it is necessary to add NBPT on a weekly basis to increase the urea concentration on a cattle feedlot surface (Varel *et al.* 1999). This becomes labour intensive and not practical. Thus, the antimicrobial chemical thymol may offer a solution to extending the activity of NBPT as shown in this study. Besides this advantage, thymol also reduces malodorous VFA production (Fig. 2b), and eliminates coliform bacteria (Table 1), which has been demonstrated previously (Varel and Miller 2001; Varel 2002), which would include *E. coli* O157:H7. Additional advantages of using thymol or possibly other plant oils are their insecticidal properties (Isman 2000; Ibrahim *et al.* 2001). We have routinely observed in our untreated cattle waste slurries numerous fly-larvae and flies, while none appear in our samples treated with thymol, carvacrol, eugenol or α-terpineol.

Obviously, field trials are needed to determine the effectiveness and economics of a combination of NBPT and thymol. It is unclear whether the additional advantages provided with thymol will justify the cost. Rough estimates to treat one metric ton of waste at 2 g kg^{-1} slurry with thymol may cost $20 (US dollars). However, we have conducted preliminary experiments with byproducts consisting of a mixture of plant oils and have estimated a cost for these in the range of $5–8 (US dollars) per metric ton of manure slurry, which includes a doubling of the concentration to 4 g kg^{-1} to treat the manure slurry. Unlike thymol, which suppresses lactate accumulation in manure slurries, these byproduct mixed oils stimulate lactate accumulation similar to eugenol (Varel and Miller 2004). Accumulation of lactate in manure slurries has a desirable effect because it is ionically a strong acid that rapidly reduces pH. This is advantageous for conserving ammonia in the waste slurry (Burgess *et al.* 1998; Smith *et al.* 2004), and would complement the NBPT additive if NBPT is not completely effective in blocking urea hydrolysis. Our efforts are ongoing to evaluate which byproduct oils are biologically and economically most effective.

Data from our third experiment which suggest that NBPT alone may prolong the viability of coliform bacteria (possibly *E. coli* and other pathogens) is a concern (Table 2). We have also observed this with swine waste slurries (J.E. Wells and V.H. Varel, unpublished data). The rationale for this occurring is supported by previous studies of Diez-Gonzalez *et al.* (2000) and Park and Diez-Gonzalez (2003). They have shown that hydrolysis of urea has antimicrobial activity by generating carbon dioxide which is trapped as carbonate. Therefore, if NBPT is used as an amendment to prevent urea hydrolysis, it also inhibits the formation of the antimicrobial compound, carbonate. These results suggest NBPT should be used in combination with another antimicrobial chemical to prevent the situation in which NBPT may promote pathogen viability. More studies are needed to clearly document this. Use of urea as a manure treatment to control patho-

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gens may be biologically effective because of the generation of carbonate and ammonia (Park and Diez-Gonzalez 2003); however, from an environmental perspective this is likely not an option.

In conclusion, our results indicate that thymol gives NBPT a supplemental effect by prolonging the inhibiting time before urea is hydrolysed in cattle manure slurries. A combination of NBPT and thymol reduces coliform bacteria and odour formation, and retains urea nitrogen in the slurry. It is possible that mixed plant oils which are less expensive when available as byproducts, could even be more practical and biologically effective than thymol. Whether or not NBPT as the sole additive to cattle manure slurries from cattle fed specific diets will prolong the viability of coliforms requires more studies.

Acknowledgements

Constructive comments and assistance of Carlos Cardenas and John Bailey (Lyondell-Millennium Chemicals) and Allen Sutton (Agrotain International) are appreciated. Technical assistance of Sue Wise and Dee Kucera, and secretarial assistance of Jackie Byrkit are also appreciated.

References

Bierman, S., Erickson, G.E., Klopfenstein, T.J., Stock, R.A. and Shain, D.H. (1999) Evaluation of nitrogen and organic matter balance in the feedlot as affected by level and source of dietary fiber. *J Anim Sci* **77**, 1645–1653.

Burgess, R.P., Carey, J.B. and Shafer, D.J. (1998) The impact of pH on nitrogen retention in laboratory analysis of broiler litter. *Poultry Sci* **77**, 1620–1622.

Burt, S. (2004) Essential oils: their antibacterial properties and potential applications in foods – a review. *Int J Food Microbiol* **94**, 223–253.

Byrnes, B. and Freney, J.R. (1995) Recent developments on the use of urease inhibitors in the tropics. *Fertil Res* **42**, 251–259.

Coates, J.D., Cole, K.A., Michaelidou, U., Patrick, J., McInerney, M.J. and Achenbach, L.A. (2005) Biological control of hog waste odor through stimulated microbial Fe(III) reduction. *Appl Environ Microbiol* **71**, 4728–4735.

Diez-Gonzalez, F., Jarvis, G.N., Adamovich, D.A. and Russell, J.B. (2000) Use of carbonate and alkali to eliminate *Escherichia coli* from dairy cattle manure. *Environ Sci Technol* **34**, 1275–1279.

Ibrahim, M.A., Kainulainen, P., Aflatuni, A., Tiilikkala, K. and Holopainen, J.K. (2001) Insecticidal, repellent, antimicrobial activity and phytotoxicity of essential oils: with special reference to limonene and its suitability for control of insect pests. *Agric Food Sci Finland* **10**, 243–259.

Isman, M.B. (2000) Plant essential oils for pest and disease management. *Crop Prot* **19**, 603–608.

McCrory, D.F. and Hobbs, P.J. (2001) Additives to reduce ammonia and odor emissions from livestock wastes: a review. *J Environ Qual* **30**, 345–355.

Mobley, H.L.T., Island, M.D. and Hausinger, R.P. (1995) Molecular biology of microbial ureases. *Microbiol Rev* **59**, 451–480.

National Research Council of the National Academies. (2002) *Final Report. Air Emissions from Animal Feeding Operations: Current Knowledge, Future Needs*. Washington, DC: National Academic Press.

Pain, B.F., Van der Weeden, T.J., Chambers, B.J., Phillips, V.T. and Jarvis, S.C. (1998) A new inventory for ammonia emissions from U.K. agriculture. *Atmos Environ* **32**, 309–313.

Park, G.W. and Diez-Gonzalez, F. (2003) Utilization of carbonate and ammonia-based treatments to eliminate *Escherichia coli* O157:H7 and *Salmonella typhimurium* DT104 from cattle manure. *J Appl Microbiol* **94**, 675–685.

Parker, D.B., Pandrangi, S., Greene, L.W., Almas, L.K., Cole, N.A., Rhoades, M. B. and Koziel, J.A. (2005) Rate and frequency of urease inhibitor application for minimizing ammonia emissions from beef cattle feedyards. *Trans ASAE* **48**, 787–793.

SAS (1996) *SAS User's Guide: Statistics*, version 6.12. Cary, NC: SAS Institute Inc.

Shi, Y., Parker, D.B., Cole, N.A., Auvermann, B.W. and Mehlhorn, J.E. (2001) Surface amendments to minimize ammonia emissions from beef cattle feedlots. *Trans ASAE* **44**, 677–682.

Smith, D.R., Moore, P.A. Jr., Haggard, B.E., Maxwell, C.V., Daniel, T.C., VanDevander, K. and Davis, M.E. (2004) Effect of aluminum chloride and dietary phytase on relative ammonia losses from swine manure. *J Anim Sci* **82**, 605–611.

Van Horn, H.H., Newton, G.L. and Kunkle, W.E. (1996) Ruminant nutrition from an environmental perspective: factors affecting whole-farm nutrient balance. *J Anim Sci* **74**, 3082–3102.

Varel, V.H. (1997) Use of urease inhibitors to control nitrogen loss from livestock waste. *Bioresour Technol* **63**, 11–17.

Varel, V.H. (2002) Carvacrol and thymol reduce swine waste odor and pathogens: stability of oils. *Curr Microbiol* **44**, 38–43.

Varel, V.H. and Miller, D.N. (2000) Effect of antimicrobial agents on livestock waste emissions. *Curr Microbiol* **40**, 392–397.

Varel, V.H. and Miller, D.N. (2001) Plant-derived oils reduce pathogens and gaseous emissions from stored cattle waste. *Appl Environ Microbiol* **67**, 1366–1370.

Varel, V.H. and Miller, D.N. (2004) Eugenol stimulates lactate accumulation yet inhibits volatile fatty acid production and eliminates coliform bacteria in cattle and swine waste. *J Appl Microbiol* **97**, 1001–1005.

Varel, V.H., Nienaber, J.A. and Freetly, H.C. (1999) Conservation of nitrogen in cattle feedlot waste with urease inhibitors. *J Anim Sci* **77**, 1162–1168.